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## FLOW REGULATION OF VASCULAR TONE

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Increases in intraluminal flow can cause both constriction and dilation. When wall tone is low, the response is constriction and when high, dilation. At one level of tone, a set-point, these effects tend to balance out. In rabbit pial arteries, when wall tone was set to different levels using serotonin and histamine and a median effective flow rate, the constrictor and dilator effects were equivalent when the smooth muscle cell membrane potential was 57.8 +- 1.1mV (n=31). When the potential was more negative than this, flow caused a slow graded depolarization of the vascular smooth muscle cell, and when more positive, hyperpolarization. Changes of tone in response to flow persist after endothelium-removal, although the magnitude of the dilator response is often much diminished. Flow-induced contraction is associated in some vessels, with the opening of voltage-dependent calcium channels and the endothelial component of dilation with activation of the inward rectifying potassium channel and EDRF release. We hypothesize that flow sensing is associated with flow (shear stress-induced) deformation of a polyanionic macromolecule. Coupling between this event and the smooth muscle responses may involve additional vasoactive factors and also electrical and mechanical mechanisms. We hypothesize that changes in intraluminal flow significantly contribute to the setting of basal muscular tone and in this way play a role in the integration of the response of a branching vascular bed to changes in pressure and tissue blood demand.

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## ROLE OF Na/Ca EXCHANGE IN REGULATING SARCOPLASMIC RETICULUM (SR) CALCIUM AND CELL RESPONSIVENESS IN VASCULAR SMOOTH MUSCLE (VSM)

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The presence and role of the Na/Ca exchanger in vascular smooth muscle (VSM) has been controversial. We employed Northern and Western blots to demonstrate, respectively, exchanger mRNA and protein in cultured rat arterial myocytes. Immuno-cytochemistry revealed that the exchanger is distributed in a reticular pattern on the VSM cell surface. This pattern may reflect co-localization with underlying junctional SR. Inhibition of the $Na^+$ pump with 1 mM ouabain for 20 min raised cytosolic $Na^+$ ($[Na^+]_{cyt}$; measured with SBFI in myocytes using digital imaging) from 4.4 to 9.0 mM. This treatment increased the cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_{cyt}$; measured with fura-2) only from 87 nM to 122 nM. Nevertheless, the ouabain treatment greatly augmented the amount of $Ca^{2+}$ in the SR stores. This was revealed indirectly by an increase in the amplitude of the $Ca^{2+}$ transients that were evoked by thapsigargin (TG; blocks the SR Ca-ATPase), serotonin and vasopressin. The increase in the


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SR $Ca^{2+}$ stores was also directly measured with chlortetracycline (CTC), a lipophilic, fluorescent, Ca-sensitive dye that can be used to measure stored $Ca^{2+}$ when the $Ca^{2+}$ concentration in the stores exceeds about $10^{-4}$ M. We observed an ouabain-induced, TG-sensitive increase in CTC fluorescence in the cultured arterial myocytes. We conclude that the Na/Ca exchanger in VSM plays an important role in regulating mobilizable SR $Ca^{2+}$ and in controlling responsiveness to vasoconstrictors.

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## THE SARCOPLASMIC RETICULAR CALCIUM PUMP CONTRIBUTES TO $Ca^{2+}$ EXTRUSION FROM VASCULAR SMOOTH MUCSLE

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We have previously reported that the release of $Ca^{2+}$ from the SR by caffeine or ryanodine, or inhibition of SR $Ca^{2+}$ accumulation by thapsigargin increases the steady state $[Ca^{2+}]_i$ in smooth muscle of the isolated rabbit inferior vena cava [1]. The increase in $[Ca^{2+}]_i$ is not accompanied by an increase in divalent cation permeability [2]. In the present report we explore the possibility that interference with SR $Ca^{2+}$ accumulation slows $Ca^{2+}$ extrusion. De-endothelialized rabbit inferior vena cava was loaded with fura-2/AM for the recording of $[Ca^{2+}]_i$ in a Spex spectrofluorimeter. After first raising $[Ca^{2+}]_i$ with a high $K^+$, high $Ca^{2+}_i$ PSS, the subsequent removal of external $Ca^+$ resulted in a decline of $[Ca^{2+}]_i$. Comparison of the above types of rates of $[Ca^{2+}]_i$ decline under different experimental conditions showed that prior SR $Ca^{2+}$ depletion by caffeine, ryanodine or thapsigargin caused a slowing of the rate of $[Ca^{2+}]_i$ decline. Control experiments established that these effects did not result from shifts in organellar $Ca^{2+}$ content, but rather were due to inhibition of $Ca^{2+}$ extrusion from smooth muscle cells. Inhibition of $Na^+/Ca^{2+}$-exchange by removal of external $Na^+$ slowed down the rate of $[Ca^{2+}]_i$ decline to a similar extent and there were no significant additive effects between external $Na^+$ depletion and thapsigargin administration. These results lead to the conclusion that SR $Ca^{2+}$ accumulation is a contributory step in $Ca^{2+}$ extrusion from vascular smooth muscle and suggest that the pathway involved consists of $Ca^{2+}$ uptake by the SR pump, vectorial release towards the plasmalemma and $Ca^{2+}$ extrusion coupled to $Na^+$ influx as proposed in the Superficial Buffer Barrier hypothesis. Calculations show that at least half of all $Ca^{2+}$ extrusion is accomplished via this pathway.

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## MYOSIN LIGHT CHAIN KINASE (MLCK) vs. LEIOTONIN

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We isolated a protein factor, leiotonin, which activated the smooth muscle actomyosin (AM) system without phosphorylating myosin light chain (MLC), but it was a proteolytic product. Subsequent effort to pursue the original leiotonin has revealed no parallelism between the actomyosin activation and MLC phosphorylation.

In the meantime, Kuwayama et al. (1988) showed that bovine stomach smooth muscle contained two types of MLCK, 155 and 130 kDa, and the former possessed about ten times stronger AM-activating effect than the latter on the basis of equivalent MLCK activity.

On the other hand, Kobayashi et al. (1992) determined the whole sequence of the 155 kDa component, which as a protein could not be distinguished from MLCK. However, this work unveiled in the N-terminal region the actin binding site which, being absent in the 130 kDa component, seemed crucial for AM activation.

Recently, we have found that wortmannin, a specific MLCK inhibitor, is a more typical agent in removing MLCK activity without reducing AM-activating effect. Beryllium sulfate affects the AM-activating effect more intensely than the MLCK activity. Thus the 155 kDa component exerts its physiological function through the mechanism not directly related to MLCK activity.

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